PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ion Transport in Plant Cells

E. A. C. MacRobbie

Phil. Trans. R. Soc. Lond. B 1971 **262**, 333-342
doi: 10.1098/rstb.1971.0099

Phil. Trans. Roy. Soc. Lond. B. **262**, 333–342 (1971) [333]
Printed in Great Britain

Ion transport in plant cells

By E. A. C. MacRobbie
Botany School, University of Cambridge

Work on ion transport in plant cells and tissues is largely concerned with the properties of cells rather than of cell layers, and the evidence is on the whole against an important role for asymmetric ion transport across cell layers equivalent to animal epithelia. Cells structurally specialized for transport, having a large increase in surface area, seem to occur singly or in small groups, but not to be organized into closely packed layers; they are known as transfer cells and were described in a wide variety of situations by Gunning & Pate (1969).

At the cell level, ion transport is best characterized in giant algal cells, but the situation may well be similar in higher plants. In *Nitella translucens* an ouabain-sensitive ATP-dependent sodium–potassium exchange pump at the plasmalemma maintains the high K/Na of the cell, and the high internal osmotic pressure is achieved by net salt uptake by a (chloride + cations) pump, also at the plasmalemma. The linkage between chloride and cations seems more likely to be chemical than electrogenic. This pump may be energized by a membrane redox system, but is not ATP-powered. The mechanism for initial entry of chloride to the cell seems also to control the distribution of tracer chloride between cytoplasm and vacuole, since the two processes of entry and transfer to the vacuole are very closely linked. The kinetics of vacuolar transfer are consistent with a pinocytotic entry of salt at the plasmalemma, fusion of pinocytotic vesicles with the endoplasmic reticulum, from which new vacuole is formed. The process of discharge to the vacuole seems to be quantized, but the mechanism and significance of this observation are not understood.

Introduction

The discussion so far has been concerned with the ion and water transporting properties of animal cells and tissues, and the purpose of this paper is to offer a reminder that plant cells exist and that they do transport ions.

The paper will be concerned very largely with a particular group of plant cells, the giant algal cells, which provide experimentally amenable material and may serve as a model for higher plant cells and tissues, more complex and experimentally less tractable. However, some general comments might first be made, by way of comparison of the ion transport processes in plant and animal tissues, and of the rather different emphasis of studies in the two.

In animal work the emphasis is firmly on the activities of epithelial cell layers, on the polarized transport of ions through such cell layers and on the consequent coupled water flows. Thus apart from the paper by Glynn, on the molecular mechanism of the sodium-potassium transport system in red cells, this meeting has been concerned with the activities of cell layers specialized for the polar transport of substances from one extracellular compartment in the animal to another. The cells of such layers have two essential properties, the provision of tight junctions preventing extracellular passage of molecules across the layer, and the existence of asymmetric processes of ion transport in their membranes, leading to polarized transfer. There is not much indication that the existence of cell layers equivalent to the animal epithelia is important in plants, and hence much more of the work on ion transport in plants is directed at the behaviour of cells, rather than that of cell layers.

There are, in plants, cell layers which might have been thought to act like animal epithelia, but the evidence is on the whole against such an equivalence. For example, the endodermis in

roots is a closely packed layer of cells separating the central cylinder of the stele from the outer cortical cell layers. In the primary state the radial cell walls of the endodermis are blocked by deposits of a fatty material, suberin, seen as Casparian bands; there is evidence that this layer serves as a barrier closing the extracellular diffusion path to solutes, and restricting movement to the symplasm, the connected cytoplasmic compartments of cortical and stelar cells with their plasmodesmatal connexions. Thus it serves the first of the functions of the animal epithelia, that of providing an effective barrier to back leakage of solutes from the stele, but the evidence is against the endodermis as the site of active transport of ions from cortex to stele. There exist in the root processes of salt transport into the xylem vessels, secretion of salts into an inert channel, with a consequent osmotic flow of water (Arisz, Helder & Van Nie 1951, Arisz 1956; House & Findlay 1966*a*), but as yet the exact location of the transport is ill-defined. Wherever the transport is sited, the coupling of water and solute flow seems to be an osmotic one, and Anderson, Aikman & Meiri (1969) suggest that the standing gradient hypothesis of Diamond & Bossert (1967) may be used to describe the flow in the xylem by which the root exudation appears at the cut end of an excised root. Studies of the transients in exudation rate produced by a change in external bathing medium (Arisz *et al.* 1951; House & Findlay 1966*b*) suggested that the site of formation of the root exudate was well inside the endodermis. There is a compartment within the root with which exchange of tritiated water is very slow (Hodges & Vaadia 1964; Anderson & House 1967), and it has been suggested that transfer to this compartment is achieved only in the transport process; if this is so, then the small size of this compartment also argues for its location within the stele (Anderson & House 1967). Thus although there is no general agreement on the site of salt transport to the xylem vessels, there is little support for a site at the endodermis, which seems to function only as a barrier separating one extra-cytoplasmic compartment from another. The same conclusion can probably be applied in other situations where an endodermis, or comparable cell layer, is to be found, in bundle sheaths in leaves or in the vascular bundles in the nitrogen-fixing root nodules in *Leguminosae*.

Animal epithelia are characterized not only by the presence of tight junctions on one side of the cell layer, but also by a great expansion of cell surface area, by the presence of microvilli and by infoldings of the membrane to form intercellular caniculi, the latter given a function in the coupling of water and solute flows in the standing gradient hypothesis. If corresponding structural arrangements exist in plant tissues, modifications specifically related to solute transport, they would seem to be the so-called *transfer cells* recognized and discussed by Gunning & Pate (1969). These are specialized cells having very large ingrowths of cell-wall material, affording a very large increase in membrane area. They are found in a wide variety of situations in higher plant tissues, but always where there is reason to believe that large solute movements occur, where the surface–volume ratio for transport is adverse, and where there is minimal associated flow of water. The function may be uptake of solute from an extra-cytoplasmic compartment or secretion to one, and the compartment may be the exterior (uptake of solutes from the environment in leaves of some water plants; secretion of salts, sugars etc. from glands of various sorts), or it may be an internal extra-cytoplasmic compartment (uptake of solutes from the xylem in leaf veins for recycling in the phloem; secretion from transfer cells into the extracellular compartment of the vascular bundles, of the amino acids and amides synthesized by the nitrogen-fixing bacteria in root nodules of leguminous plants). The last of these examples is interesting in that it is suggested (Pate, Gunning & Briarty 1969) that the endodermis of the bundle acts as a barrier with tight junctions, but the transfer cells provide the site for active

transport. Transfer cells are also characterized by the presence of many mitochondria and a great deal of endoplasmic reticulum, closely associated with the wall ingrowths; Gunning & Pate (1969) have suggested that the endoplasmic reticulum may be important as a transport compartment, particularly since it extends through the plasmodesmata. There is as yet no direct experimental determination of solute fluxes through transfer cells, but there is enough presumptive evidence for a specialized function in transport to suggest that their role deserves careful study.

In contrast to the emphasis on epithelia in animal work on ion transport, most of the plant work is directed to the activities and behaviour of cells rather than of tissues. One reason for this may simply be that it is difficult to explain the activities of cells in plant tissues, without a clear understanding of the details of the behaviour of its individual cells, when nearly every cell in the tissue has a large central sap cavity occupying some 90 % of the cell volume. In ion movement through a tissue, each cell vacuole acts as a diversionary sink competing with the next cells on the path for the ions in transit through the cytoplasm, and it is therefore essential to understand the movement of ions within the individual plant cell, if we are to study movement through a plant tissue.

In the study of ion transport processes in individual cells we may aim to answer a series of questions, on the existence of active transport processes within the plant cell and their location at specific membranes of the cell, on the nature of the energy supply for the active transport and of its coupling to the ion movement, and on the detailed molecular mechanism of the ion transfer across the membrane. The existence of active ion transport processes in a very wide range of plant cells has been established by measurements of ion concentrations and potentials, although their certain localization at specific membranes demands separate measurements of cytoplasmic and vacuolar concentrations and is only possible, as yet, in the giant algal cells. However, it is clear that many plants cells, of very diverse kinds, are capable of maintaining both a very high K/Na ratio relative to that in the environment, and of accumulating salt from a very low external concentration to a very high internal level, thereby generating a very considerable internal osmotic pressure and consequent turgor. It seems that net salt accumulation from the environment is *one* of the ways in which a large central sap cavity of high osmotic pressure may be maintained. In giant algal cells, in which the central sap cavity is filled with sodium and potassium chlorides it appears to be *the* way, but in higher plant cells other vacuolar constituents and secretion processes are common. Given potassium chloride in the medium the cell will accumulate both ions in the vacuole; given potassium sulphate externally the cell will effect a K^+/H^+ exchange at the plasmalemma, and a secretion of potassium with an internally produced organic acid anion into the vacuole; in the absence of external salt the cell may secrete sugars or organic acids into the vacuole. The pattern found depends, among other things, on the conditions of growth and the age of the tissue. Steward & Mott (1970) have argued that the process of vacuolation, the separation of an internal volume by a process of internal solute secretion, is primary, and that salt uptake is only one facet of this, to be regarded as such rather than to be accorded a significance of its own. Hence although we may start with the idea of transfer of ions across a preformed membrane it may well be that the problem of net salt accumulation is in fact one of the creation of vacuole, of the continuous formation of new small vacuoles, which then fuse with the pre-existing vacuole.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Ion transport in giant algal cells

Plasmalemma fluxes

There are four freshwater species on which a good deal of work has been done, namely *Nitella translucens*, *Chara corallina* (formerly *australis*), *Tolypella intricata*, and *Hydrodictyon africanum*. The first three are Characean species, having cylindrical internodal cells perhaps 10 cm long and 700 to 1200 μm in diameter; in these the cytoplasm forms a peripheral layer about 10 μm thick, of which the outer 5 to 6 μm is a stationary gel layer with the chloroplasts embedded in it in regular array, and the inner 5 to 6 μm is free of chloroplasts and streams at about 50 μm s^{-1} parallel to the chloroplast array. *Hydrodictyon africanum* has giant spherical cells, 3 to 6 mm in diameter, derived from a colonial net of 256 or 512 genetically identical cells.

Figures for ion concentrations and potentials in a large number of giant cells are given in a recent review (MacRobbie 1970*a*), and the discussion here will be restricted to these four species, with strong emphasis on *Nitella translucens*. All four species demand active influx of chloride and active efflux of sodium, but whereas *Nitella* and *Hydrodictyon* demand also an active influx of potassium, in the other two species potassium is close to its equilibrium value and could be maintained by purely passive fluxes. The same two patterns of ion pumps are in fact found in a much wider range of plant cells which have been investigated. Measurements of cytoplasmic ion concentrations make it clear that both the high K/Na and high Cl concentration of the cell are developed across the plasmalemma, although further active transport between cytoplasm and vacuole may also be required, of Na and probably Cl in *Nitella*, of Na and Cl in *Chara*, and probably of K, Na and Cl in *Tolypella*. In addition, the high salt concentration in the cytoplasm as a whole, and the requirement for osmotic equilibrium with the vacuole, demands accumulation within the chloroplasts, and further regulation of the K/Na balance at the chloroplast membranes is also indicated. Direct measurements of ion concentrations in non-aqueously extracted chloroplasts from *Tolypella* confirm this (Larkum 1968). Hence ion pumping activity seems to be required at every membrane for which we have measurements, and the list is unlikely to be complete.

The conclusions from measurements of concentration and potential are confirmed by study of the ion fluxes under a wide range of conditions (of light, temperature, external concentration, presence of inhibitors of various more or less specific kinds). (References in full are given in MacRobbie 1970*a*, 1971; see also Raven 1968*a*.) It appears that *Nitella* and *Hydrodictyon* are basically similar, and that in both species the plasmalemma fluxes may be partitioned into three components:

(1) A cation exchange pump responsible for a component of K influx, and a component of Na efflux which depends on the presence of potassium in the outside solution. This pump is light-dependent, sensitive to ouabain, and sensitive to uncouplers. The linkage between sodium efflux and potassium influx, and the sensitivity to ouabain suggest a common mechanism with the pump so well known in animal cells.

(2) A (chloride + cations) transport system. This is responsible for the influx of chloride, but also has associated cation influxes, the components of K and Na influx which depend on the presence of chloride in the outside solution, under conditions in which the chloride pump can function. This transport system is strongly light-dependent but is insensitive to ouabain and relatively insensitive to uncouplers. The nature of the linkage between the chloride influx and the associated cation influxes is not certain; the linked components of sodium and potassium

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

influx make up only 30 to 70 % of the increment of chloride influx produced by the addition of monovalent cations to a calcium chloride solution, and hence we must suppose the balance is made up by a chloride-linked H^+ influx, or by an anion exchange process. The small potential changes observed on removing chloride from the solution (Spanswick, Stolarek & Williams 1967) make it unlikely that the coupling between chloride and cations is electrogenic, even if the observed dependence of ion fluxes on membrane potential, which is much steeper than the Goldman equation predicts (Walker & Hope 1969), is used to calculate the expected potential change. Hence unless local hyperpolarizations, and current circulations in the membrane, are envisaged the evidence seems to favour a chemical coupling of chloride and cations, a pump for salt as such.

(3) Residual components of cation influxes and effluxes, chloride efflux. These presumably represent the 'passive' fluxes through the membrane, but they account for relatively small fractions of the total fluxes.

Both transport systems are strongly light-dependent (although both can function at a very reduced rate in the dark), but the nature of the coupling seems to be very different in the two cases. There are three ways in which partial reactions of photosynthesis in the chloroplast could be used to drive an ion transport process in the plasmalemma (Robertson 1968)—by the provision of ATP from photosynthetic phosphorylation to a membrane ATPase, or of a reduced compound of some kind of a membrane redox system, or of hydroxyl ions arising from light-dependent pH shifts across the chloroplast membranes, to be used to drive ion exchange reactions in the plasmalemma. By the use of various wavelengths of light, capable of driving only cyclic phosphorylation, or of the complete photosynthetic electron transfer chain with production of both ATP and reducing power, and by the use of inhibitors of various partial reactions of photosynthesis or respiration, it is possible to measure ion transport under conditions where ATP but not reducing power can be produced. In both *Nitella* (MacRobbie 1965, 1966*a*; Smith 1967), and *Hydrodictyon* (Raven 1967), it seems that whereas ATP is an adequate energy supply for the cation transport, the chloride transport seems to be independent of phosphorylation. This conclusion is particularly well-established in *Hydrodictyon*, by a very extensive series of studies of light and inhibitor effects (Raven 1969*a*, *b*). Hence although the nature of the energy coupling for the chloride transport is still uncertain, it is clearly not ATP, in contrast to the cation transport or to most animal transport systems. Possible mechanisms are discussed in MacRobbie (1970*a*).

Although the patterns of ion transport in *Nitella* and in *Hydrodictyon* are very similar, that found in *Chara corallina* differs in important respects. *Chara* does not appear to have an active influx of potassium, and its active sodium efflux, though present, is not sensitive to ouabain. The active chloride influx is in general light-sensitive, and inhibited by the removal of monovalent cations, although in some cells these effects are less marked (Findlay *et al.* 1969). In contrast to the results in *Nitella* and *Hydrodictyon*, the chloride influx in *Chara* is as sensitive to uncouplers as is photosynthetic CO_2 fixation and there is no evidence which is inconsistent with ATP as the energy source (Smith & West 1969); the effects of different wavelengths of light on chloride influx in *Chara* are not known, but the difference between such similar species is surprising and deserves further study.

Two other ion pumps in giant algal cells should be mentioned. Active uptake of bicarbonate has been studied in *Hydrodictyon* (Raven 1968*b*) and in *Nitella* (Smith 1968), and appears to be powered in the same way as the chloride uptake, but to be capable of working at a consider-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

ably higher rate. An active extrusion of H^+ in *Nitella clavata* was postulated by Kitasato (1968), and acidification of the solution outside parts of the cell was observed by Spear, Barr & Barr (1969) and by Smith (1970). Very little is known of the metabolic connexions of this transport, but the postulated rates are very high. Spear *et al.* (1969) suggest that this is the primary transport process in the cell, and that chloride influx is a secondary consequence, arising from a build-up of H^+ in the membrane and a back-flow into the cell of molecular HCl. Smith (1970) also suggests a primary function for the H^+ extrusion, and postulates uptake of KCl as a result of a dual ion-exchange process, H^+/K^+ annd OH^-/Cl^-.

Transfer of ions to the vacuole

There is evidence from studies of the distribution of tracer between cytoplasm and vacuole during uptake which suggests that chloride entry at the plasmalemma may not be a process of single ion transfer across the membrane, but a process which also determines the subsequent intracellular distribution of the tracer (MacRobbie 1969).

The simplest model for the cell, which proves to be inadequte, is that of two intracellular phases, cytoplasm and vacuole, in series with one another. The kinetics of such compartmental systems are discussed in MacRobbie (1971) and will be given only briefly here. On the simple 2-phase model the cytoplasmic specific activity during uptake will rise initially on a (1-exp) time-course, characterized by a single rate constant k, determined by the ratio of the fluxes out of the cytoplasm (to vacuole and to outside), to the cytoplasmic content. The amount of activity in the vacuole should show an initial lag, the rate of transfer to the vacuole rising slowly as the cytoplasm fills; the percentage of total cell activity which is in the vacuole should rise linearly with time, from the origin. In the first experiments (MacRobbie 1966*b*) the description appeared to be adequate, and the rate constant k was found to be proportional to the influx. (We may notice what this means; if cell A has twice the chloride influx of cell B, then after uptake for a given time the amount of tracer in the vacuole of cell A is four times that in cell B; as the influx increases, a correspondingly higher fraction of the higher total entry is transferred to the vacuole.) In subsequent experiments, looking at the early part of the time course, it was found that the behaviour was more complex. Figure 1 shows the time course of appearance of tracer chloride in the vacuole; there is no initial lag (i.e. it is less than 1 min), and the graph of the percentage in the vacuole, although linear, does not go through the origin. Hence there are two components of chloride transfer to the vacuole, a fast component linear with time (seen as the intercept in figure 1*a*), and a second slower component which involves a cytoplasmic phase filling with a (1-exp) time course (the rising part of the graph in figure 1*a*). That the fast component is not simply cytoplasmic contamination of the vacuolar samples is shown by the double-labelling experiment of figure 2, in which ^{42}K and ^{36}Cl influx were measured in the same cells; the fast component is present for chloride but not for potassium. It was therefore argued that the results show that the cytoplasm is heterogeneous with respect to its ion exchange, and that two distinct cytoplasmic compartments are involved. Tracer chloride reaching the vacuole via the fast phase equilibrates with only a very small fraction of the total cytoplasmic chloride. The problem is then to identify the two compartments with structural phases of the cytoplasm. An upper limit can be set on the content of the fast phase from the shortness of the lag period, and this then can be compared with the measured chloride content of the chloroplast layer, and of the flowing cytoplasm. The chloride content of the cytoplasmic layer is about 200 nmol cm^{-2} (MacRobbie 1964), and of the flowing cytoplasm about 33–44 nmol cm^{-2} (Spanswick &

Williams 1964; Hope, Simpson & Walker 1966). Under high flux conditions the upper limit to the content of the fast phase was calculated to be 17 nmol cm^{-2}, but under low flux conditions a more stringent condition, with an upper limit on the content of only 3 nmol cm^{-2} applies. Hence the fast compartment can represent only a very small fraction, even of the content of the flowing cytoplasm.

The most curious feature of the results is the very close link, in both phases of exchange, between entry of chloride to the cell and transfer to the vacuole. Thus comparing mean values of groups of cells in different experimental conditions, we find a constant fraction of the total

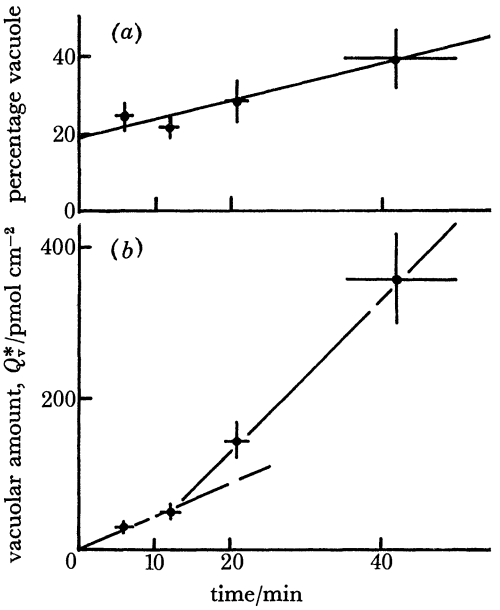

FIGURE 1. (*a*) Percentage of the total activity in the cell which is in the vacuole after various times of uptake of ^{36}Cl. (*b*) Amount of tracer chloride in the vacuole. In each group each point shows the mean value for five to seven cells, with its standard error, over the range of uptake times shown.

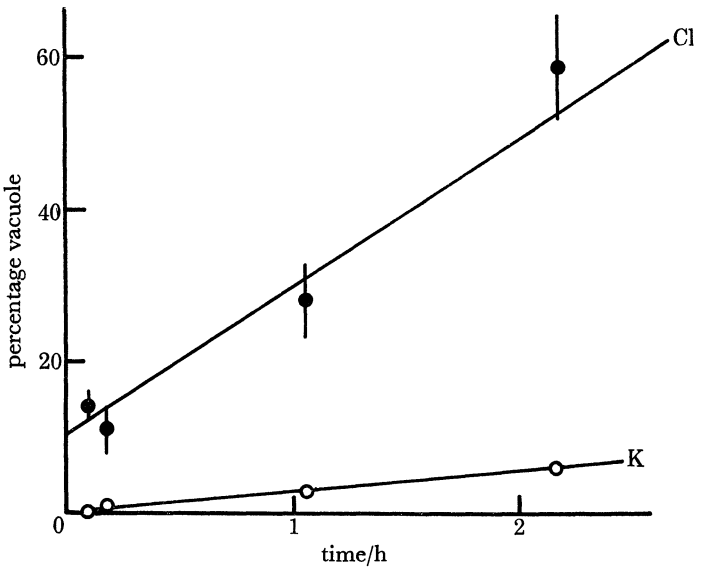

FIGURE 2. Percentage of the total cell activity found in the vacuole after various labelling times; double labelling of the same cells with ^{42}K and ^{36}Cl.

entry in the fast fraction, and also that the exchange between fast and slow components is proportional to influx. In the experiments described the rate of transfer to the vacuole, as a fraction of the influx to the cell, was equal to 0.13 plus about 0.08 per nmol cm^{-2} of tracer chloride in the cytoplasm. The important point is that the level of activity in the cytoplasm does not specify the rate of transfer to the vacuole directly, but only the rate as a fraction of the influx. Influx and transfer to the vacuole are so intimately linked that we can predict only their ratio at any given level of label in the cytoplasm, and not the absolute value of vacuolar flux. We can answer a question in either of the following forms: 'How much activity is present in the cytoplasm of a cell when there is x % of the total activity in the vacuole?' or 'How much when the instantaneous rate of transfer to the vacuole is y % of the total influx?'; but we cannot specify either the absolute amount in the vacuole at any stage during uptake, or the absolute rate of transfer of tracer to the vacuole, unless we know the influx. It appears therefore that the distribution of tracer between cytoplasm and vacuole is controlled by the influx. (The word 'controlled' is used rather than 'correlated' because of the wide range of external conditions under which this relation applies, including for example changes of external chloride which are unlikely to affect metabolism.) It was suggested that this curious property could be achieved if salt entered the cell by a process involving the formation of salt-filled vesicles at the plasmalemma, which moved through the cytoplasm with some exchange of ions on the way, to discharge either directly at the tonoplast, or by fusion with the endoplasmic reticulum from which new vacuole was subsequently formed. This is pinocytosis of a type, but clearly an indiscriminate uptake of all small molecules in the outside solution is not involved, and some form of selective adsorption on the membrane must precede the membrane flow. Active transport by means of membrane flow and vesiculation was suggested by Bennett (1956), and discussed by Sutcliffe (1962); recently Hall (1970) has suggested that vesicles derived from the plasmalemma by pinocytosis can be identified in the cells of maize root tips, and that their number increases under conditions in which the cells are accumulating salt.

In a second paper (MacRobbie 1970*b*) the relations between apparent influx to the vacuole in the fast component of the time course (M_{ov}) and the total influx of chloride to the cell (M_T) were examined, in the individual cells in a single experiment, rather than in the means of groups of cells under different conditions. It was found that the values of M_{ov}/M_T (i.e. Q^*_v/Q^*_T, the percentage activity in the vacuole) were not normally distributed, but fell into groups. The means of the groups were, within the errors of the experiment, in the ratios of 1:2:3, with some higher values (in *Nitella* collected from the wild 80 % of the cells, and in *Nitella* cultured in the laboratory 95 % of the cells, fell into these three groups). For example, in one experiment on cultured *Nitella*, eight cells had 14 to 34 % of the total activity in the vacuole, with a mean of 28 ± 2 %, twelve cells had 44 to 70 % of the activity in the vacuole with a mean of 53 ± 2 %, three cells had 76 to 86 %, with a mean of 80 %, and there were no cells between or outside these ranges. The range of influx values in each group was similar, and the mean influx values were 1.5 ± 0.2, 1.4 ± 0.1, and 1.7 pmol $cm^{-2}\ s^{-1}$ respectively, i.e. the total influx is the same in each group. This means that the cells fall into non-overlapping groups in which the mean percentages in the vacuole are in the ratio $1.0 \pm 0.07 : 2.0 \pm 0.08 : 3.0$, but the mean influxes are in the ratio $1.0 \pm 0.1 : 0.9 \pm 0.09 : 1.1$. The results of six experiments on collected cells, six experiments on cultured cells, and a further four experiments on collected cells are shown in figure 3; the conclusion is the same in every case. Thus partition of the cells into groups based on their vacuolar percentages effects no partition into groups of

different mean influx values. This is only possible if M_{ov} and M_T are correlated but the cells fall into distinct groups (otherwise cells having a low value of M_{ov}/M_T will have higher than average M_T, cells having a high value of M_{ov}/M_T will have a lower than average M_T, and the full range of M_T values will be found only in the middle group of intermediate values of the ratio M_{ov}/M_T). That the vacuolar percentage ratios in the groups fall close to the *same* ratio of 1:2:3 in each experiment in figure 3 could not arise by chance. It seems there-

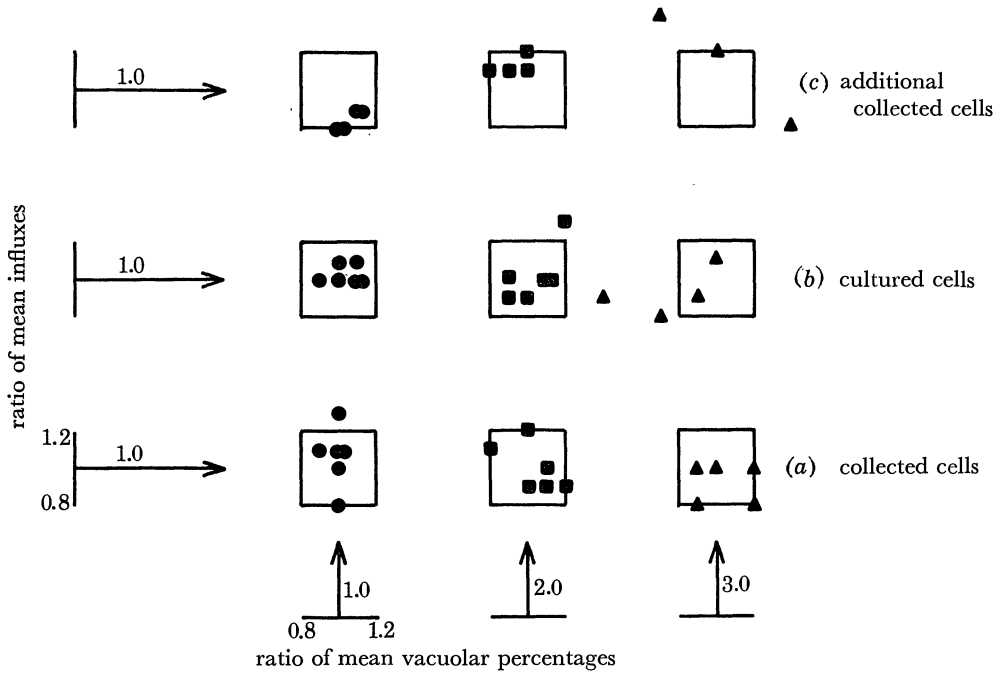

FIGURE 3. Separation of cells in each experiment into groups having the same mean influx but mean vacuolar percentages in the ratio of 1:2:3. (*a*) Collected cells, results from MacRobbie (1970*b*). Six experiments, 98 cells in these groups. (*b*) Cultured cells, results from MacRobbie (1970*b*). Six experiments, 107 cells in these groups. (*c*) Additional experiments on collected cells. Four experiments, 62 cells in these groups. The boxes show the ranges 0.8 to 1.2 for influx ratios, and 0.8 to 1.2, 1.8 to 2.2, and 2.8 to 3.2 for vacuolar percentage ratios. The standard errors of the means are 6 to 31 % of the mean value in the group for influx, and 2 to 11 % of the mean value in the group for vacuolar percentages.

fore that the component of rapid transfer to the vacuole is linearly related to the influx of chloride to the cell, but is quantized; M_{ov} is a quantized fraction of the total influx. This implies that the flux from the fast compartment of the cytoplasm to the vacuole is quantized, but is also very closely related to the influx (since it is the ratio M_{ov}/M_T which is quantized and not the flux M_{ov}). It was argued that this must mean that entry to a cytoplasmic phase precedes a quantized discharge from this phase to the vacuole, and that the endoplasmic reticulum is the most likely intermediary phase. This corresponds with what many electron microscopists believe to be the origin of the vacuole, from the endoplasmic reticulum, and some evidence for this is also available in *Nitella* (Costerton & MacRobbie 1970). Thus the rapid entry phase may consist of two distinct structural components, a mobile system of pinocytotic vesicles and the endoplasmic reticular network with which these rapidly fuse. (The network in *Nitella* consists of extensive cisternae in the peripheral cytoplasma, profiles extending inwards between the chloroplasts and an anastomosing network of tubules in the streaming cytoplasm.)

The system is obviously complex, and as yet, ill-understood, particularly the significance of

the quantization. But the kinetics observed place severe restrictions on the type of process likely to be involved, and seem impossible to explain in terms of fixed cytoplasmic compartments. Some form of dynamic membrane system, in which new vacuole is created, seems to be required rather than a process of single ion exchange across a preformed membrane. It seems likely that in the ion accumulation in the vacuole of these giant cells, well past the stage of rapid expansion, we are nevertheless looking at the same processes as are responsible for the initial formation of vacuoles, at processes of continuing vacuolation.

The giant algal cells may not provide a perfect model for the more complex activities of higher plant cells, but they do provide a system in which we can design feasible experiments and frame meaningful questions, and in which the underlying transport activities may not be entirely dissimilar. When we understand the giant algal cells (which is clearly not yet) we may then be in a position to consider whether the same processes do occur in higher plant cells. For ion transport studies at a cell level, as distinct from a tissue level, the giant algal cells provide the only experimental systems we can cope with, using present experimental methods.

References (MacRobbie)

Anderson, W. P., Aikman, D. P. & Meiri, A. 1970 *Proc. Roy. Soc. Lond.* B **174**, 445–458.
Anderson, W. P. & House, C. R. 1967 *J. exp. Bot.* **18**, 544–555.
Arisz, W. H. 1956 *Protoplasma* **46**, 5–62.
Arisz, W. H., Helder, R. J. & Van Nie, R. 1951 *J. exp. Bot.* **2**, 257–297.
Bennett, H. S. 1956 *J. biophys. biochem. Cytol.* **2**, 94–103.
Costerton, J. W. F. & MacRobbie, E. A. C. 1970 *J. exp. Bot.* **21**, 535–542.
Diamond, J. M. & Bossert, W. H. 1967 *J. gen. Physiol.* **50**, 2061–2083.
Findlay, G. P., Hope, A. B., Pitman, M. G., Smith, F. A. & Walker, N. A. 1969 *Biochim. biophys. Acta* **183**, 565–576.
Gunning, B. E. S. & Pate, J. S. 1969 *Protoplasma* **68**, 107–133.
Hall, J. L. 1970 *Nature, Lond.* **226**, 1253–1254.
Hodges, T. K. & Vaadia, Y. 1964 *Plant Physiol.* (*Lancaster*) **39**, 104–108.
Hope, A. B., Simpson, A. & Walker, N. A. 1966 *Aust. J. biol. Sci.* **19**, 355–362.
House, C. R. & Findlay, N. 1966*a* *J. exp. Bot.* **17**, 344–354.
House, C. R. & Findlay, N. 1966*b* *J. exp. Bot.* **17**, 627–640.
Kitasato, H. 1968 *J. gen. Physiol.* **52**, 60–87.
Larkum, A. W. D. 1968 *Nature, Lond.* **218**, 447–449.
MacRobbie, E. A. C. 1964 *J. gen. Physiol.* **47**, 859–877.
MacRobbie, E. A. C. 1965 *Biochim. biophys. Acta* **94**, 64–73.
MacRobbie, E. A. C. 1966*a* *Aust. J. biol. Sci.* **19**, 363–370.
MacRobbie, E. A. C. 1966*b* *Aust. J. biol. Sci.* **19**, 371–383.
MacRobbie, E. A. C. 1969 *J. exp. Bot.* **20**, 236–256.
MacRobbie, E. A. C. 1970*a* *Q. Rev. Biophys.* **3**, 251–294.
MacRobbie, E. A. C. 1970*b* *J. exp. Bot.* **21**, 335–344.
MacRobbie, E. A. C. 1971 *A. Rev. Plant Physiol.* **22**, (in the Press).
Pate, J. S., Gunning, B. E. S. & Briarty, L. G. 1969 *Planta, Berl.* **85**, 11–34.
Raven, J. A. 1967 *J. gen. Physiol.* **50**, 1627–1640.
Raven, J. A. 1968*a* *Abh. dt. Akad. Wiss. Berl.* **4***a*, 145–151.
Raven, J. A. 1968*b* *J. exp. Bot.* **19**, 193–206.
Raven, J. A. 1969*a* *New Phytol.* **68**, 45–62.
Raven, J. A. 1969*b* *New Phytol.* **68**, 1089–1113.
Robertson, R. N. 1968 *Protons, electrons, phosphorylation and active transport.* Cambridge University Press.
Smith, F. A. 1967 *J. exp. Bot.* **18**, 716–731.
Smith, F. A. 1968 *J. exp. Bot.* **19**, 207–217.
Smith, F. A. 1970 *New Phytol.* **69**, 903–917.
Smith, F. A. & West, K. R. 1969 *Aust. J. biol. Sci.* **22**, 351–363.
Spanswick, R. M. & Williams, E. J. 1964 *J. exp. Bot.* **15**, 193–200.
Spanswick, R. M., Stolarek, J. & Williams, E. J. 1967 *J. exp. Bot.* **18**, 1–16.
Spear, D. G., Barr, J. K. & Barr, C. E. 1969 *J. gen. Physiol.* **54**, 397–414.
Steward, F. C. & Mott, R. L. 1970 *Int. Rev. Cytol.* **28**, 275–370.
Sutcliffe, J. F. 1962 *Mineral salts absorption in plants.* Oxford: Pergamon.
Walker, N. A. & Hope, A. B. 1969 *Aust. J. biol. Sci.* **22**, 1179–1195.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES